**REMARKS**

By this amendment, claims 1 and 9 have been amended, claims 12-15 have been added, and claim 7 has been cancelled without prejudice. Claims 1, 3-6, and 9-15 are thus currently under examination in the present application. For the reasons set forth below, Applicants submit that the present amendments and arguments place this application in condition for immediate allowance.

As an initial matter, with regard to new claims 12-15, Applicants respectfully submit that the addition of these claims is proper and that these claims should be entered as no new matter has been added by their addition. Specifically, new claim 12 has simply been added to further recite a particular method of generating antibodies where the recited method generates antibodies for assaying certain fuel oxygenates and where X is a spacer comprising a hydrocarbon chain of 2-8 carbon atoms. Both of these features were initially presented in claims 3 and 7 of the application, as filed. Similarly, new claims 13 and 14 were merely added to incorporate alternative dependencies that were initially presented in claims 4 and 6 of the application, as filed. Finally, new claim 15 has been added to further recite a particular method for assaying a sample for certain fuel oxygenates by immunoassay techniques. Support for that amendment can be found, for example, on pages 16-17 and throughout the specification of the application, as filed. Accordingly, no new matter has been added by the addition of claims 12-15.

In the Office Action of March 30, 2009, the Examiner first objected to claim 7 for being of improper dependent form. This objection has now been rendered moot by virtue

of the present amendments which cancel claim 7 without prejudice, and thus the objection should be withdrawn.

In the Office Action dated March 30, 2009, the Examiner then made various rejections to claims 1, 3-7, and 9-11 under 35 U.S.C. §112, second paragraph as being indefinite. In particular, the Examiner asserted that certain terms and phrases in claims 1 and 9 where unclear. Each of these rejections is addressed in detail below and, accordingly, for the reasons set forth below, Applicants submit that the Examiner's rejections are respectfully traversed and should be withdrawn.

First, with regard to the Examiner's rejection of claim 1 as being indefinite under 35 U.S.C. §112, second paragraph, the Examiner has continued to assert that that the terms "spacer" and "groups capable of binding to a carrier protein" were indefinite because it was unclear what compounds or groups could be encompassed by those terms. Contrary to the Examiner's assertions, however, both of these terms, as well as the concepts they represent, are routinely used in the art and would thus be readily understood by those of ordinary skill in the art, including chemists and biochemists.

As shown in the attached Declaration of David C. Cullen, Ph.D. pursuant to 37 C.F.R. §1.132[1], the terms "spacer" and "groups capable of binding to a carrier protein" are known in the art, and scientists in the field are, in fact, very familiar with the use of spacers and groups capable of binding to a carrier protein in procedures where a molecule, such as a hapten, is attached to a protein. Indeed, the term "spacer" is widely used in organic chemistry to refer to a flexible part of a molecule that provides a

[1] The executed copy of this Declaration will follow shortly.

connection between two other parts of a molecule. See attached Wikipedia entry for "Molecular Spacer." Furthermore, when a spacer is attached to a carrier protein for the purpose of raising antibodies, it is known that interposing a spacer between a hapten and a protein commonly improves the ability of the conjugate to elicit the production of antibodies that are specific to the small molecules. In these situations, the nature of the spacer itself may have some effect on its ability to elicit antibody production. However, it is an entirely simple and routine matter to test various spacers of differing lengths in different conjugates and then determine which spacer achieves the optimal effect for a particular application. See attached Declaration of David C. Cullen at para. 4. As shown in portions of the book "Bioconjugate Techniques," which are discussed in the Declaration of David C. Cullen submitted in conjunction with this response and which are attached to the Declaration as Exhibits A-C, various techniques for producing hapten-protein conjugates by using suitable spacers have, in fact, been known for a number of years.

As a further example of the use of spacers to conjugate haptens to proteins and the routine nature of trying a range of spacers to achieve an optimal effect for a particular application, the abstract of Yoo, et al., which was submitted in conjunction with the previous response in this application, describes that "five haptens of fenthion differing in spacer arm length (4-8) atoms were synthesized...and conjugated to bovine serum albumin and keyhole limpet hemocyanin to be used as immunogens." Similarly, the abstract of Shinkaruk, et al., which was also submitted in conjunction with the previous response in the application, includes a similar description and refers to the attachment of

haptens via spacer arms "with the length of the spacer arms being three or four carbon atoms" while concluding that "specificity is not influenced by the length of the spacer arm."

As yet another example of the use of the term "spacer" when referring to haptens, the attached article of Santos Riccardi, et al. states in the discussion on page 5 of that article that "[d]ifferent types of spacer groups were used to synthesize the immunogen and the tracer...[to] help to avoid problems associated with antibodies directed to the spacer group rather than to the hapten itself." The article of Santos Riccardi, at al. goes on to indicate that '[i]n some cases the use of heterological spacer improves the sensitivity of the assay" and that "[t]he haptens with C6 spacer arms conjugated to [horseradish peroxidase] resulted in 3-5 fold increased sensitivity compared to haptens with C3 spacer arms." As such, and in light of the references to the term "spacer" in the foregoing references, it is thus the case that the meaning of the term "spacer" would be readily understood by one of ordinary skill in the art and that one of ordinary skill in the art would readily understand the types of molecules that are encompassed by the term "spacer."

Similarly, with regard to the term "group capable of binding to a carrier protein," it is also the case that that term is entirely clear to one of ordinary skill in the art. In this regard, it is further noted that in the introduction section of the attached Santos Riccardi, et al. article, there is a reference to "a functional group capable of covalent bonding to [an] enzyme" and it is indicated that "[c]ommon functional groups are –COOH, -OH, -$NH_2$, or –SH." As one of ordinary skill in the art would recognize, these types of

functional groups are precisely what is being referred to in the present application by the use of the term "group capable of binding to a carrier protein" because attaching a molecule to a protein via these various groups is an entirely routine matter. See also the attached Declaration of David C. Cullen, para. 8 and Exhibit B.

Accordingly, in light of the foregoing discussion regarding the use of the term "spacer" and the phrase "group capable of binding to a carrier protein," it is thus clear that these terms are routinely used in the art when referring to haptens and thus create no indefiniteness issues whatsoever under 35 U.S.C. §112, second paragraph.

With regard to the Examiner's rejection of claim 9 under 35 U.S.C. §112, second paragraph, the Examiner asserted that claim 9 is indefinite because the immunoassay steps and the intended use of the immunoassay was unclear. Further, the Examiner also asserted that the term "breakdown products" in claim 9 was indefinite because it was unclear what compounds are encompassed by the term "breakdown products." By the present amendments, claim 9 has been amended to recite further positive steps for carrying out the immunoassay and to indicate that the immunoassay is being employed for determining the amount of fuel oxygenates and their respective breakdown products in a sample. Support for these amendments can be found, for example, on pages 16-17 and throughout the specification of the present application, as filed.

Additionally, by the present amendments, claim 9 has also been amended to further recite particular fuel oxygenates, which were initially presented in claim 7 of the application as filed. As such, one of ordinary skill in the art would readily be apprised of

the breakdown products of these particular fuel oxygenates and would understand exactly what compounds are encompassed by the term "breakdown products."

In light of the discussion above, Applicants thus submit that the claims of the present application are in compliance with 35 U.S.C. §112, second paragraph. Accordingly, Applicants respectfully submit that the Examiner's rejections under 35 U.S.C. §112, second paragraph are respectfully traversed and should be withdrawn.

In the Office Action dated March 30, 2009, the Examiner then further rejected claims 1, 3-7, and 9-11 under 35 U.S.C. §112, first paragraph as lacking enablement. Specifically, the Examiner asserted that while the specification was enabling for generating antibodies against methyl tert-butyl ether, ethyl tert-butyl ether, methyl tert-amyl ether, and tert-butyl alcohol, the specification did not reasonably provide enablement for generating antibodies against all fuel oxygenates. Without addressing the merits of the Examiner's assertion, this rejection has now been rendered moot by virtue of the present amendments to claims 1 and 9, which have been amended to refer to particular fuel oxygenates selected from methyl tert-butyl ether, ethyl tert-butyl ether, methyl tert-amyl ether, and tert-butyl alcohol. Accordingly, Applicants thus submit that the Examiner's rejection, insofar as applied to the claims as amended, is respectfully traversed and should be withdrawn.

In the Office Action, the Examiner next rejected claims 1, 3-7, and 9-11 under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. Specifically, the Examiner asserted that the terms "spacer" and "group capable of binding to a carrier protein" are not defined in the specification and that the

specification does not provide adequate guidance in terms of what compounds or structures are encompassed by those terms. For the reasons set forth below, Applicants submit that the Examiner's rejections are respectfully traversed and should be withdrawn.

As described above in relation to the Examiner's rejection of the claims under 35 U.S.C. §112, second paragraph, the terms "spacer" and "group capable of binding to a carrier protein" are well known in the art , and thus one of ordinary skill in the art would readily be apprised of the specific structures encompassed by those terms. Indeed, the prior art is replete with numerous examples of the types of "spacers" that can be used to attach a carrier protein to a hapten, and it is simply a routine matter to try a range of spacers of different lengths when conjugating a carrier protein to a hapten in order to achieve an optimal effect. See, e.g., the abstracts of Yoo, et al. and Shinkaruk, et al., the attached article by Santos Riccardi, et al., and the attached Declaration of David C. Cullen, which are each discussed in detail above.

In the Office Action, the Examiner referred to an article by Issert, et al. (Amino Acids, 1999) to support an assertion that "conjugating a carrier for a small molecule through different linkers (spacers) provides different immunogenic responses." See Office Action dated March 30, 2009 at pages 7-8. In Fig. 3 of that article, however, the results of experiments involving four rabbits are shown, where two of the rabbits were injected with a conjugate involving one spacer and two of the rabbits were injected with a conjugate involving a different spacer. Upon analysis of the results from these experiments, the authors observed that the two rabbits injected with one of the conjugates gave both the best and the worst results, whereas the two rabbits injected with the other

conjugates both yielded similar, intermediate results. As such, it is thus clearly not the case that conjugating a carrier to a molecule using different linkers will necessarily provide for a different immunogenic response.

The aim of the overall process, using a hapten conjugated to a protein via a spacer, is simply to produce antibodies to the hapten, and the spacer is merely the tool that is used to facilitate the antibody production. The spacer does not affect the nature of the antibodies produced, but can influence the efficiency with which they are produced. As such, one of ordinary skill in the art, who would readily understand the different molecules that are encompassed by the term "spacer," could easily test a variety of spacers to determine which spacer would achieve the optimal effect and could easily do so without undue experimentation.

Furthermore, it is also noted that the phrase "group capable of binding to a carrier protein" is well known in the art, and thus one of ordinary skill in the art would readily understand the various molecules that are encompassed by that phrase. As described above with respect to the Santos Riccardi, et al. article, the introduction of that article refers to a functional group that is capable of covalently binding to an enzyme and then goes on to indicate that common functional groups include –COOH, -OH, $-NH_2$, or –SH. As such, this thus further demonstrates that attaching a molecule via " a group capable of binding to" a protein is an entirely routine matter to one of ordinary skill in the art and would require no undue experimentation. Indeed, as also set forth in the attached Declaration of David C. Cullen, one of ordinary skill in the art would readily understand

which groups could be utilized to attach a particular molecule to a protein, as he would already be aware of numerous suitable groups.

Accordingly, in light of the foregoing discussion, Applicants submit that the claims of the present application are in full compliance with the written description requirement of 35 U.S.C. §112, first paragraph. Thus, Applicants respectfully submit that the Examiner's rejections under 35 U.S.C. §112, first paragraph are respectfully traversed and should be withdrawn.

Finally, in the Office Action, the Examiner rejected claim 7 under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,416,671 ("Pourfarzaneh"). In particular, the Examiner asserted that, although Pourfarzaneh does not disclose antibodies that bind to compounds related to methyl tert-butyl ether, Pourfarzaneh discloses an antibody capable of binding to methyl-tert butyl ether and it would be expected that the antibody would bind to compounds similar to methyl-tert butyl ether. By the present amendments, this rejection has now become moot by virtue of the cancellation of claim 7 without prejudice and, accordingly, the Examiner's rejection should be withdrawn.

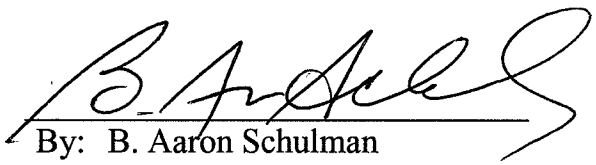
Nevertheless, it is noted that, in the Office Action, the Examiner stated that "Claim 7 is drawn to a monoclonal antibody according to the method of claim 1..." Previously, however, claim 7 was directed to a method according to claim 1. In other words, claim 7 was previously directed to a method of generating antibodies, and was not directed to a monoclonal antibody. Pourfarzaneh does not teach or even remotely suggest a method by which antibodies could be obtained. Instead, Pourfarzaneh simply describes the use of a methyl tert-butyl ether mouse monoclonal antibody and provides no direction whatsoever

as to where or how such an antibody may be obtained. Accordingly, Applicants further respectfully submit that the claims of the present application are not rendered obvious by the cited Pourfarzaneh reference.

In light of the amendments and arguments provided herewith, Applicants submit that the present application overcomes all prior rejections and objections, and has been placed in condition for allowance. Such action is respectfully requested.

Respectfully submitted,

Date: September 30, 2009



By: B. Aaron Schulman
Registration No.: 31,877

**STITES & HARBISON PLLC**◆1199 North Fairfax St◆Suite 900◆Alexandria, VA 22314
TEL: 703-739-4900 ◆ FAX: 703-739-9577 ◆ CUSTOMER NO. 000881

# APPENDIX

# Molecular spacer

From Wikipedia, the free encyclopedia

A **Molecular spacer** or simply a **spacer** in chemistry is any flexible part of a molecule providing a connection between two other parts of a molecule.

Retrieved from "http://en.wikipedia.org/wiki/Molecular_spacer"
Categories: Molecular geometry

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# Preparation and evaluation of atrazine immunoconjugate

## Preparo e avaliação do imunoconjugado para atrazina

**Carla dos Santos Riccardi[I]; Ângela Regina Araújo[II];Hideko Yamanaka[I]**

[I]Departamento de Química Analítica - Instituto de Química - UNESP - 148-01-970 - Araraquara - SP
[II]Departamento de Química Orgânica - Instituto de Química - UNESP - 148-01-970 - Araraquara - SP

**ABSTRACT**

In order to study the affinity reaction between the anti-atrazine antibody and atrazine, an enzyme was incorporated, as a marker, to an atrazine carboxylic derivative. The hapten and conjugate were synthesized and characterized by MS, IR and NMR. The interaction between monoclonal antibodies and hapten-HRP conjugate was investigated by enzyme linked immunosorbent assay (ELISA).

**Keywords:** atrazine, conjugate, ELISA, environmental monitoring

**RESUMO**

A fim de estudar a reação de afinidade entre os anticorpos anti-atrazina e a atrazina, uma enzima foi incorporada, como marcador, a um derivado carboxílico da atrazina. O hapteno foi caracterizado por espectometria de massa (EM), infravermelho (IV) e ressonância magnética nuclear (RMN). A interação entre anticorpos monoclonal e o conjugado de hapteno-HRP foram determinados pelo ensaio imunoenzimático (ELISA).

**Palavras-chave:** atrazina, conjugado, ELISA, monitoramento ambiental

## Introduction

The initial delineation stages of an immunoassay involve the selection of the target molecule; preparation of the hapten, which consists in the synthesis of the target molecule derivative containing a group for attachment to the protein; covalent binding of the hapten with enzyme to form a hapten-enzyme conjugate; and finally the affinity reaction.

The ideal hapten for a selected target analyte molecule has to be a nearperfect mimic of that molecule, both in structure and geometry, in electronic and hydrogen-bonding capabilities, and in hydrophobic properties. The hapten should contain a "handle", terminated with a functional group capable of covalent bonding to enzyme. Common functional groups are -COOH, -OH, $-NH_2$ or $-SH$[3]. The small hapten molecules require to the spacer group between enzyme and hapten molecule in order to improve the recognition sites with antibody.

## Materials and methods

### Chemicals

Isopropylamine, *N, N*-diisopropylethylamine, cyanuric chloride (2,4,6-trichloro-1,3,5-triazin), *N*-hydroxysuccinimide (NHS), *N, N*-dicyclohexylcarbodiimide (DCC), Tween 20, bovine serum albumin (BSA) and peroxidase (HRP) (EC 1.11.1.7) type VI-A from horseradish, 1100 U/mg were purchased from Sigma; 6-aminohexanoic acid, anhydrous dimethylformamide (DMF) and ethylenediaminetetracetic acid (EDTA) were purchased from Merck. Other reagents were analytical grade.

The monoclonal antibodies (mAb, clone K4E7) were given by Prof. Dr. B. Hock, Technical University of München, Freising.

### Apparatus

Mass spectra (MS) was obtained on a VG-Platform-Fisons spectrometer (VG Analytical, Wythenshawe, U. K) using 70-eV ESI for ionization and data are reported as m/z (relative intensity). Infrared spectra (IR) was measured on a FTIR Perkim Elmer 2000 spectrometer. $^1H$ and $^{13}C$: NMR spectra were obtained with a General Electric AC 200 F (Bruker NMR, Billerica, MA) opering at 200 MHz for $^1H$ and 75 MHz for $^{13}C$. Chemical shifts ($\delta$) were expressed in parts per million using tetramethylsilane as an internal standard. Thin Layer Chromatography (TLC) was performed on 0.25 mm, pre-coated silica gel 60 F254 aluminum (Merck, Gillstown, NJ). Compounds were detected by exposure to iodine vapor; eluent systems were described in the individual experiments. ELISA (Enzyme Linked ImmunoSorbent Assay) was performed in 96-well microplates (COSTAR) with Organon Teknika 2001 reader.

### Synthesis of Hapten

Hapten (Hp) was synthesized by the routes as shown in Figure 1, following the procedure described by Goodrow et al.[2]. The resulting hapten was characterized by MS, IR, and $^1H$ and $^{13}C$:NMR.

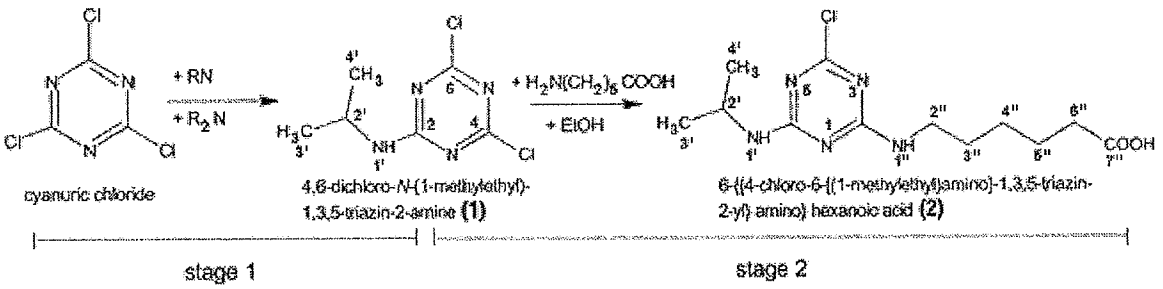


**FIGURE 1** – Synthesis and structure of atrazine carboxylic derivative for conjugation to a linked enzyme.

To 50.0 mmol of cyanuric chloride in 400 mL of ether cooled to - 20$^0$C was added, over 45 min, 52.0 mmol of isopropylamine (70 wt% in water) and 52.0 mmol of *N, N*-diisopropylamine in 50 mL of ether. The mixture was filtered, and the filtrate was washed sequentially with 1 mol $L^{-1}$ HCl (25 mL), 5 % m/v $NaHCO_3$ (25 mL), and satured NaCl (2 x 25 mL) and then dried ($Na_2SO_4$). The ether was removed under reduced pressure, leaving a pale yellow oil (Figure 1, compound **1**). This compound reacted with 6-aminohexanoic acid (20.0 mmol) and the compound **2** (Figure 1) was obtained. The purity of both compounds were investigated by TLC (Thin-Layer Chromatography, chloroform/methanol 1:1 v/v).

### Preparation of the enzyme tracer

The hapten-HRP conjugate was synthesized using the method described by Pradelles[5]. 20 µL of *N*-hydroxysuccinimide (200 nmol) and 20 µL *N, N*-dicyclohexylcarbodiimide (200 nmol), both dissolved in anhydrous dimethylformamide (DMF), were successively added to 20 µL of atrazine carboxylic derivative solution in DMF. After incubation for 4h at room temperature, 60 µL of this mixture was added to 600 µL of HRP (1 mg $mL^{-1}$) dissolved in 0.1 mol $L^{-1}$ borate buffer solution (pH 8.5). The reaction was processed for 30 min at room temperature and then it was stopped by the addition of 400 µL of 0.1 mol $L^{-1}$ phosphate buffer solution (pH 7.4) containing 0.4 mol $L^{-1}$ NaCl, 1 mmol $L^{-1}$ EDTA, 0.1 % m/v bovine serum albumin and 0.01 % m/v sodium azide. The obtained mixture was dialyzed for 12h against 0.01 mol $L^{-1}$ borate buffer solution (pH 8.5) and 4h against 0.03 mol $L^{-1}$ phosphate buffer solution (pH 7.5). Aliquot of the Hp-HRP were stored frozen.

## Elisa (Enzyme Linked ImmunoSorbent Assay) development

The Hp-HRP conjugate was evaluated by ELISA. The 96-well microtiter plates were coated with 100 µL mAb specific for atrazine, prepared in 50 mmol $L^{-1}$ carbonate buffer solution (pH 9.6), at 4 $^0$C overnight. The plates were washed three times with phosphate buffered saline (PBS)/Tween 20 solution (50 mmol $L^{-1}$ pH 7.6, 0.05 % m/v Tween 20). Then the plates were incubated for 1h with PBS/Tween 20 solution containing 0.1 % m/v BSA. After a second similar washing step, 100 µL enzyme tracer were added and incubated for 1h at 37 $^0$C. Sequentially the plates were washing and

100 μL substrate solution (3, 3', 5, 5'-tetramethylbenzidine and ureia peroxide) were added. The enzymatic reaction processed for 20 min. Then were added 100 mL reaction stop solution (1 mol $L^{-1}$ sulfuric acid) to each well and the absorbance were measured at 450 nm. All steps were carried out at room temperature except for mAb coating and affinity reaction stages.

## Results and discussion

### Hapten Synthesis

The development of immunoassays and the preparation of haptens for atrazine or other *s*-triazines has been well documented [1,2,4,6]. Different types of spacer groups were used to synthesize the immunogen and tracer. This can help to avoid problems associated with antibodies directed to the spacer group rather than to the hapten itself. In some cases the use of heterological spacers improves the sensitivity of the assay. The haptens with C6 spacer arms conjugated to HRP resulted in 3-5 fold increased sensitivity compared to the haptens with C3 spacer arms. In general, HRP tracers with the long spacer arms resulted in the most favorable assay conditions. This hapten has a C6 spacer that mimics the ethyl group of the atrazine. At the same time, important antigenic determinants in the atrazine chemical structure such as the bulky isopropylamine group and the electronegative chlorine atom remains distant from the shielding effect caused by the protein [2].

The synthesized compounds were detected on TLC by staining in an iodine chamber. TLC Rf 0.51 e 0.13 to compounds **1** and **2** (white pasty, mp 160-161 $^0$C), respectively. Structural confirmation was accomplished by MS, IR, $^1$H and $^{13}$C: NMR (Proton-Desacopled $^{13}$C and Distortionless Enhancement by Polarization Transfer (DEPT) experiments). All of these date were consistent with the assigned structure for the compounds 1 and 2. From the mass spectra it was observed *m/z* 206 and 302, together with the peaks [M + 1] e [M + 2] regarding to the presence halide in the molecule (Cl isotopic). IR (KBr) bands had been found ν N-H (3400 / 3450 $cm^{-1}$), δ N-H (1620 / 1580 $cm^{-1}$), ν C-H (< 3000 $cm^{-1}$) and ν C-Cl (745-695 $cm^{-1}$) (compounds **1** e **2**, respectively); ν C=O (1698 $cm^{-1}$) (compound **2**). The presence of the atrazine carboxylic derivate was confirmed by $^1$H and $^{13}$C:NMR (Table 1), and also by direct comparison with the respective literature data [2]. The $^1$H: NMR interpretation was initiated by analysis of the alifatics chains of compound **1** (*N*-alkyl-amino group) and compound **2** (*N*-alkyl-aminohexanoic group). Finally, $^{13}$C:NMR experiments confirmed the presence of the carbon atoms of the heterocycle and hexanoic acid chain.

**TABLE 1** – $^1$H-NMR Data of Compounds **1** ($CDCl_3$, 200MHz) and **2** (DMSO-$d_6$, 200MHz)[2].

| Carbon | 1 | | 2 | |
|---|---|---|---|---|
| | $^1$H | $^{13}$C | $^1$H | $^{13}$C |
| | $\delta_H$ mult J | $\delta_C$ | $\delta_H$ mult J | $\delta_C$ |
| 2 | | 166.00 | | 165.40 |
| 4 | | 168.50 | | 169.04 |
| 6 | | 164.83 | | 164.50 |
| 1' | 7.27 sl | | 7.60 brs | |
| 2' | 4.23 m | 43.70 | 3.99 m | 42.98 |
| 3' | 1.24 d (6.6Hz) | 21.80 | 1.12 d (6.4Hz) | 21.65 |
| 4' | 1.26 d (6.6Hz) | 21.94 | 1.14 d (6.4Hz) | 21.96 |
| 1'' | | | 7.80 brs | |
| 2'' | | | 2.19 m | 33.59 |
| 3'' | | | 1.50 m | 24.14 |
| 4'' | | | 1.30 m | 26.59 |
| 5'' | | | 1.50 m | 28.34 |
| 6'' | | | 3.20 m | 39.80 |
| 7'' | | | | 174.43 |
| -OH | | | 11.90 | |

## Elisa

On these experiments it was observed the ability of mAc (K4E7) to a binding with hapten-HRP conjugate. The linear range of 25-200 μg $mL^{-1}$ (Figure 2) was verified for the conjugate concentrations by interaction with the monoclonal antibodies (10 μg $mL^{-1}$). Above of this value, the enzymatic immunoreaction tends to the saturation of the bonds sites by monoclonal antibodies (exponencial response: 200-240 μg $mL^{-1}$).

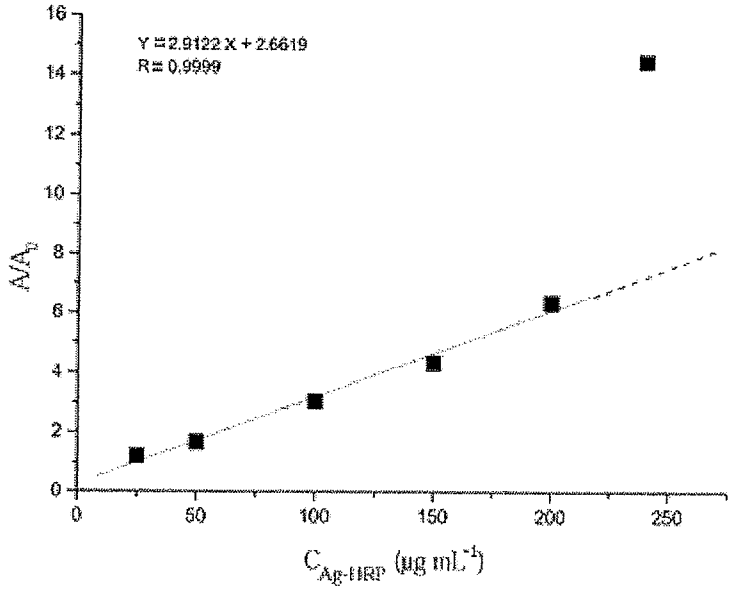


**FIGURE 2** – Analytical curve of ELISA used to evaluate the binding of Hp-HRP conjugate. Microplates coated with fixed concentration of the antibodies 10 $\mu g\ mL^{-1}$ were exposed to differents concentrations of conjugated.

## Conclusions

The atrazine derivate was convenient synthetized and the hapten-HRP conjugate was suitable for the recognition with the anti-atrazine antibodies. Further, it will be used for the amperometric immunosensor investigation.

## Acknowledgement

We thank Prof. Dr. B. Hock for giving the monoclonal antibodies; Prof. Dr. P. I. Costa, for helping on the immunoassays investigation; Department of Organic Chemistry by disposing the equipments for the compounds characterization and FAPESP for the fellowship.

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